

Pharmaceutical Development of Calcium and Vitamin K table – New Technique for treatment of Cancer

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Abstract: In the present study an attempt was made to formulate and evaluate Calcium and Vitamin K₃ tablet using wet granulation technique incorporating various Excipients like Starch Powder, Starch Paste, Magnesium Sterate, Aerosil, Talc, Lactose, Vitamin K Liquid form and Water. The Formulated tablets were evaluated for different physicochemical properties like rheological properties, weight variation, thickness, hardness, % friability, *In vitro* release studies and drug content. Studies revealed that all the physicochemical parameters comply with the official standards. The *in vitro* release studies exhibits the release up to 90%, over a prolonged period of time which confirms the extended release profile of formulation, having better bioavailability as well as decreased dosing frequency with reduced doses.

Key Words: Calcium, Vitamin K, Formulation, tablet, anticancer, tab.

Key messages: Calcium and Vitamin K₃ combination possess chemo preventive action. It suppresses the tumor lesions and decreases the biochemical markers which are elevated in HCC. Formulation of this remarkable drug will open new perspectives that Calcium and Vitamin K₃ combination may prevent slow or treat the occurrence of liver cancer reducing the cost of HCC.

INTRODUCTION:

It is well established that variations in cytosolic calcium concentration $[Ca^{2+}]_c$ trigger key cellular functions, for example, contraction of myofilaments, secretion of hormones and neurotransmitters and modulation of metabolism, to cite a few. ^[1, 2] Moreover, Ca^{2+} also has a major function in triggering mitotic division in numerous cell types (e.g., T lymphocytes and of oocytes) and, conversely, in the regulation of cell death. ^[3] The notion that the cellular Ca^{2+} overload is highly toxic, causing massive activation of proteases and phospholipases was known to cell biologists since the late 1960s. Electron micrographs of clearly damaged cells showed swollen mitochondria full of Ca^{2+} phosphate precipitates in the 1960s and 1970s and the toxicity of Ca^{2+} ionophores in cultured cells was one of the first effects of these molecules to be described. ^[4] Classically, this toxic role of Ca^{2+} has been associated to

necrosis, that is, the catastrophic derangement of cell integrity and function following the exposure to different types of cell injury and leading to the activation of Ca^{2+} -activated hydrolysing enzymes. Typical examples are complement-induced cell death and excitotoxicity, in which glutamate-dependent hyper stimulation leads neurons to the necrotic death.^[5]

More recent data, however, have suggested a function of Ca^{2+} also in the regulation of other types of cell death. Several studies have shown that the increases of $[\text{Ca}^{2+}]$ occur, both at early and late stages of the apoptotic pathway^[6] and both Ca^{2+} release from the endoplasmic reticulum (ER) and capacitative Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} channels have been proposed to be apoptogenic.^[2] Thus, a common view is that while severe Ca^{2+} dysregulation can promote cell death through necrosis, more controlled intracellular $[\text{Ca}^{2+}]$ increases induced by milder insults promote cell death through apoptosis.

The question obviously arises as to how a single-signaling molecule can trigger, often in the same cell type, so vastly the different functions. The key to solving this puzzle is presumably in the unique physicochemical characteristics of Ca^{2+} and its capacity to establish local concentrations within cells that in turn form a variety of recognizable signatures corresponding to specific functional effects. Indeed, a unique characteristics of Ca^{2+} within the cell cytoplasm is its low rate of diffusion (compared with the other classical second messengers, cyclic AMP, cyclic GMP and inositol 3-phosphate (IP₃), diffusion of Ca^{2+} is over 100-fold slower), due to the presence of a variety of binding sites.^[2] Also, in distinction to the other messengers, several organelles disseminated throughout the cell can sequester Ca^{2+} and, in response to appropriate signals, release it back into the cytoplasm. These conditions are ideal for the generation of subcellular heterogeneities in $[\text{Ca}^{2+}]$, for example, in the proximity of plasma membrane or organelle Ca^{2+} channels. The existence of subcellular domains in which $[\text{Ca}^{2+}]$ largely exceeds the bulk cytosolic values have been postulated for a long time, but only in the last decade, these values have been directly measured and their primary function in controlling some cell functions, beyond the classical function in promoting neurosecretion at the presynaptic membrane, has become evident.^[2]

Vitamin K₃ derivatives have been shown to exert anticancer activities. Here we show a novel vitamin K₃ derivative (S)-2-(2-hydroxy-3-methyl butylthio) naphthalene-1, 4-dione, which is named as CR108 that induces apoptosis and tumor inhibition through reactive oxygen species (ROS) and mitochondrial dysfunction in human breast cancer. CR108 is more effective on the breast cancer cell death than other vitamin K₃ derivatives.

Moreover, CR108 induced apoptosis in both the non-HER-2-overexpressed MCF-7 and HER-2-overexpressed BT- 474 breast cancer cells. CR108 caused the loss of mitochondrial membrane potential, cytochrome c released from mitochondria to cytosol, and cleaved PARP proteins for apoptosis induction. CR108 markedly increased ROS levels in breast cancer cells. N-acetylcysteine (NAC), a general ROS scavenger, completely blocked the CR108-induced ROS levels, mitochondrial dysfunction and apoptosis. Interestingly, CR108 increased the phosphorylation of p38 MAP kinase but conversely inhibited the surviving protein expression. NAC treatment prevented the activation of p38 MAP kinase and rescued the surviving protein levels. SB202190, a specific p38 MAP kinase inhibitor, recovered the surviving protein levels and attenuated the cytotoxicity of CR108-treated cells.

Furthermore, CR108 inhibited the xenografted human breast tumor growth in nude mice. Together, we demonstrate that CR108 is a novel vitamin K₃ derivative that induces apoptosis and tumor inhibition by ROS production and mitochondrial dysfunction and associates with the phosphorylation of p38 MAP kinase and the inhibition of survivin in the human breast cancer.

Vitamin K consists of a family of structurally similar fat-soluble 2-methyl-1,4- naphthoquinones, including phyloquinone (vitamin K₁), menaquinone (vitamin K₂), and menadione (vitamin K₃).^[7]

Phylloquinone and menaquinone are natural products but menadione is a synthetic analog of vitamin K.^[7] Vitamin K3 contains potent anticancer effects against various types of carcinoma cells, including breast, hepatic, oral cavity, pharyngeal, mammary and bladder.^[7]

Vitamin K3 has been shown to cause apoptosis in the human breast cancer cells via a mitochondria-related pathway.^[8] The main effect of vitamin K3 against cancer was due to oxidative stress via redox-cycling of the quinone to produce reactive oxygen species (ROS). Quinones can undergo either one-electron reduction to produce semiquinone radicals or two-electron reduction for resulting in hydroquinones.^[9] Vitamin K3 induced single- and double-strand DNA breaks via ROS generation in breast cancer cells.^[10] Combination with vitamin K3 and conventional chemotherapeutic agents showed the synergistic effects *in vivo* and *in vitro*.^[10,11] Moreover, pretreatment with vitamin K3 before doxorubicin or mitomycin increased cytotoxicity to breast cancer cells.

Several novel vitamin K3 derivatives have been shown anticancer activities. For example, vitamin K3 analogs induced selective tumor cytotoxicity in the neuroblastoma cells.^[12] 2-(2-Hydroxyethylsulfanyl)-3-methyl-1,4-naphthoquinone (Cpd 5), a vitamin K3 analog, is a potent growth inhibitor for hepatoma cells. Protein tyrosine phosphatases (PTPases) such as the dual specificity phosphatases CDC25A are potential target proteins for Cpd 5.^[13] Besides, 2-(2-hydroxy-ethylsulfanyl)-3-methyl-5-nitro-1,4-naphthoquinone (PD-37) and 2-(2-hydroxy-ethylsulfanyl)-3-methyl 5-acetylamino-1,4-naphthoquinone (PD-42) are synthesized vitamin K3 analogs, which have similar anticancer activities to Cpd 5.^[14]

MATERIALS AND METHODS:

Calcium and Niacin was received as a gift sample from Elder Pharmaceuticals Pvt. Ltd, Dehradun (India). Starch Powder, Starch Paste, Magnesium Sterate, Aerosil, Talc, Lactose, Vitamin K Liquid form and Water was procured from Elder Pharmaceuticals Pvt. Ltd, Dehradun (India). All the chemicals were of analytical grade.

Preparation of Calcium and Vitamin K3 (LP) Tablets:

The weights of all the ingredients were checked. Calcium and Vitamin K3 was sifted through Suitable size. Mannitol and Pregelatinised maize starch was sieved through appropriate sieve. Milling of Ferric oxide red was done with starch maize to obtain particular group size. Soon after that calcium and Vitamin K3, was blended with Mannitol and Pregelatinised maize starch, Milling Ferric oxide red with starch maize in a Rapid mixer granulator for 2-5min.

Wet Granulation was done with blended material ACE Inhibitor (LP), Calcium hydrogen phosphate, Mannitol and Pregelatinised maize starch, Milling Ferric oxide red with starch maize using Binder solution in a Rapid mixer granulator for 2-5min.

Wet Granulated material was dried at 40°C in a Fluid bed drier. Water content of the main was filled between 1.5 to 3.5% w/w. The dried granules were sieved again through appropriate sieve. Pregelatinised maize starch and Magnesium stearate was passed through appropriately sieve separately. Sifted Pregelatinised maize starch was added to the dried granules and then mix for 10 min in a non-shear blender and then magnesium stearate was added and mixed for 5-7 min.

The approved blend was compressed using approved tooling of 6-8 mm circular flat bevelled in process quality control must monitor the compression to ensure compliance to current approved in process specification for compressed tablet.

Environmental Condition:

Die punch was fixed on compression machine as per the following size and shape.

Die size and shape=8.0mm, round shaped; Punch size and shape- Upper Punch=8.0mm, round flat bevelled punch embossed with 10. Lower punch=8.0mm, round flat bevelled punch, scored.

De-duster was fixed and metal detector to the discharge chute of the compression Machine.

Load the Granules to the hopper of compression Machine. (Table 1, 2)

Evaluation of Formulation:

Determination of Hardness of Tablets:

Randomly 12 tablets from the batch of formulation were used for the determination of hardness with the help of Dr.Schleuniger'spharmatron Hardness tester. The sample mean, X_{\max} and X_{\min} were calculated.

Determination of Weight variation:

Ten tablets selected at a random were weighed accurately and the average weights of the tablet were calculated. Then the deviation of individual weight from the average weight and the standard deviation were calculated.

Determination of Thickness of tablets:

The individual crown-to-crown thickness of ten tablets was determined by using Vernier calliper for each batch. The sample mean and standard deviation of each tablet were calculated.

Determination of friability of Tablets:

Dedust the tablets carefully and weigh accurately the required number of tablets (20 tablets).

Place the tablets in the drum and rotate it 100 times. Remove the tablets; remove any loose dust from them accurately.

Formula:- $W_1 - W_2 \times 100 / W_1$

Determination of Disintegration of Tablets:

Apparatus-The apparatus consist of a basket-rack assembly, a 1 litre beaker a thermostatic arrangement for heating the fluid and a mechanical device for raising and lowering the basket in the immersion fluid at a constant frequency rate

Method- Unless otherwise stated in the individual monograph, introduce 1 tablet into each tube and if directed in the appropriate general monograph, add a disc to each tube. Suspend it.

The assembly in the beaker containing the specified liquid and operate the apparatus for the specified time. Remove the assembly from the liquid. The tablets pass the test if all of them have disintegrated.

RESULTS:

Fluid Uptake v/s Hardness of Formulation F1

Hardness of tablets when compared with fluid intake has been accessed for the formulation F1. [Table 3], [Figure 1]

Fluid Uptake on Hardness of Formulation F1 and F2

Hardness of tablets when compared with fluid intake has been investigated for the formulation F1 and F2. [Table 4], [Figure 1]

Friability Test

Friability test has been accounted using (20 tablets /100 rev). [Table 5], [Figure 2, 3]

Parameter

Friability (NMT 0.5%W/W)

$(W_1 - W_2) \times 100 / w_1$

Disintegration test

Disintegration v/s Hardness test has been accessed for both the formulations F1and F2 Formulations. [Table 6], [Figure 3, 4 and 5]

Parameter

Disintegration time (NMT -7min)

Thickness [Vernier Callipers]

Effect of thickness on Formulation F1 and F2. [Table 7], [Figure 6].

Parameter

Thickness (3.4 - 4.0 mm)

Effect of Hardness v/s %Drug release, *In vitro* study

Invitro studies have been done to evaluate the relation and efficiency of hardness and drug release. [Table 8] [Figure 7, 8]

DISCUSSION:

Two Formulation F1 and F2 were prepared by using the wet granulation method. In both the formulation (F1 and F2) all the ingredients were in same ratio but the solvent (water) Quantity have been changed in both F1 and F2 Formulation.

In F1 Formulation Quantity of Vitamin K (Liquid Form) 13%

In F2 Formulation Quantity of Vitamin K (Liquid Form) 15%

The effect of water amount were analyzed in both the Formulations. [Table 1, 2, 3]

Hardness

Dr. Scheuniger Hardness tester was used to evaluate the hardness of tablets. In F1 formulation the hardness crosses the limits i.e. 50-100N where as in F2 formulation hardness was found within the range of 50-100N. [Table 3, 4], [Figure 1]

Weight Variation

The weight variation study was performed using 15 individual randomly selected samples from each batch. The weight uniformity results of prepared tablets indicate no significant difference from the average value.

Friability

The friability study was performed using Roche friability apparatus on 10 individuals randomly selected sample from each batch. The friability results of prepared samples indicate significant difference and major variation between F1 and F2 formulation. [Table 2, 3], [Figure 2, 3]

Disintegration

The disintegration study was performed on disintegration apparatus using 4 individuals randomly selected sample from each batch (F1 and F2) whereas the F1 formulation crosses the limits i.e. NMT 7min and the disintegration time of F2 formulation were found within the range. [Table 6] [Figure 3, 4 and 5]

Thickness

The thickness study was performed using Vernier calliper apparatus on 10 randomly selected samples from each batch. The thickness of F1 formulation was found to be below the limits i.e. (3.4-4mm) where as F2 formulation was found within the range. [Table 7], [Figure 6].

***Invitro* Study**

The *invitro* release of Calcium and Vitamin K3 formulation from different batches was studied using USP 11 type dissolution apparatus. The prepared batches showed a significance variation on the results depending upon the Quantity of solvent used.

In F1 formulation majority of formulated tablets were below the limits i.e. (90-100%) whereas the F2 formulation were within the limits.

FIGURES:

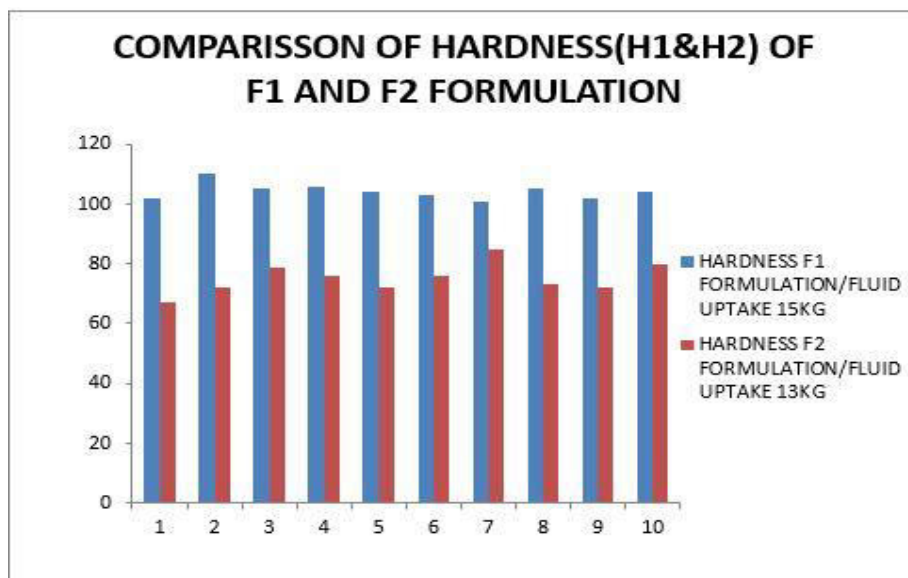


Figure 1: Effect of Fluid Uptake on Hardness of Formulation F1 and F2

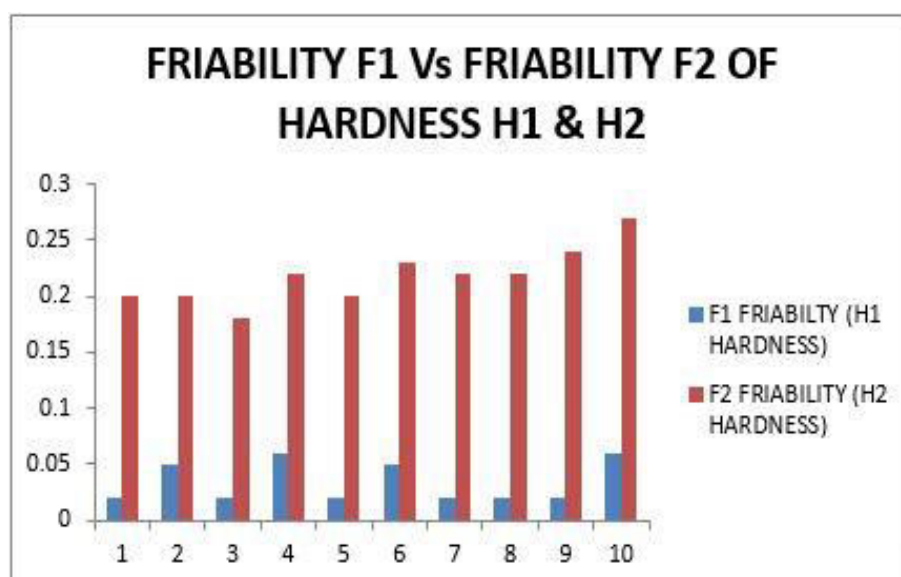


Figure 2: Effect of Friability on hardness on Formulations.

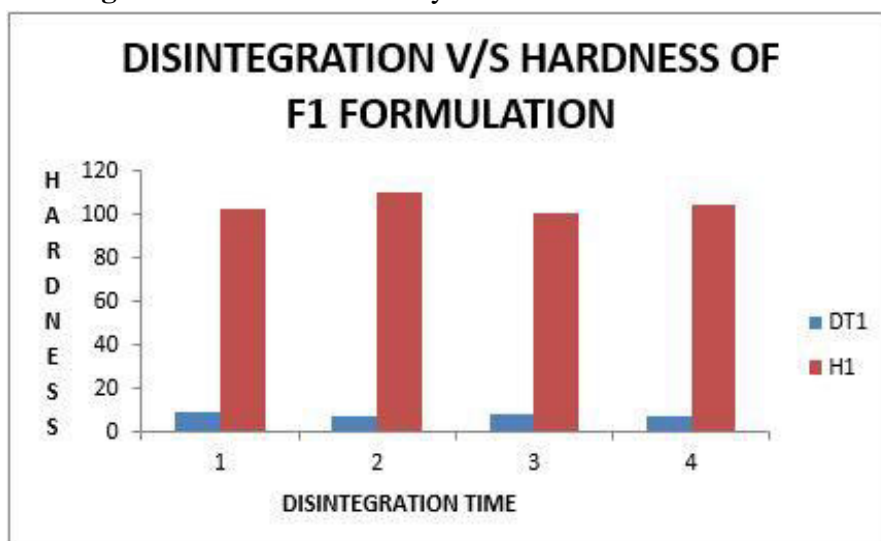


Figure 3: Formulation Graph Plot Disintegration v/s Hardness of F1 Formulation

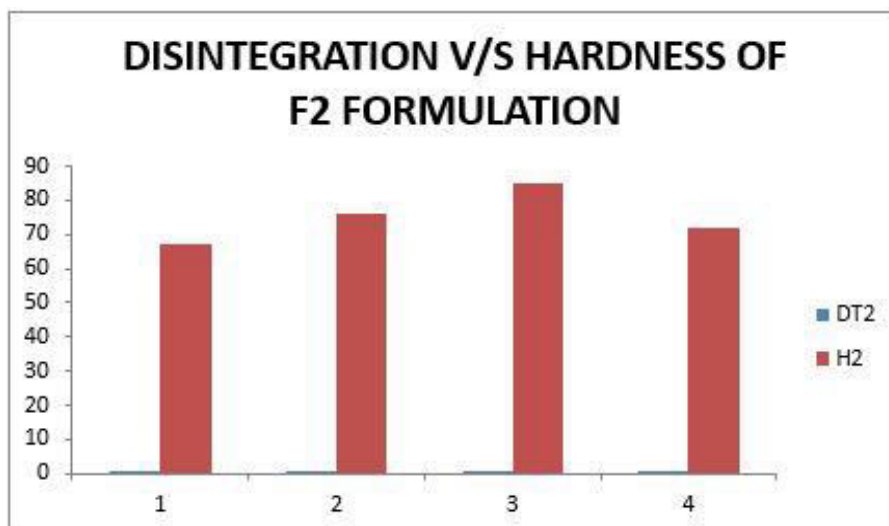


Figure 4: Graph Plot Disinteration v/s Hardness Of F2 Formulation

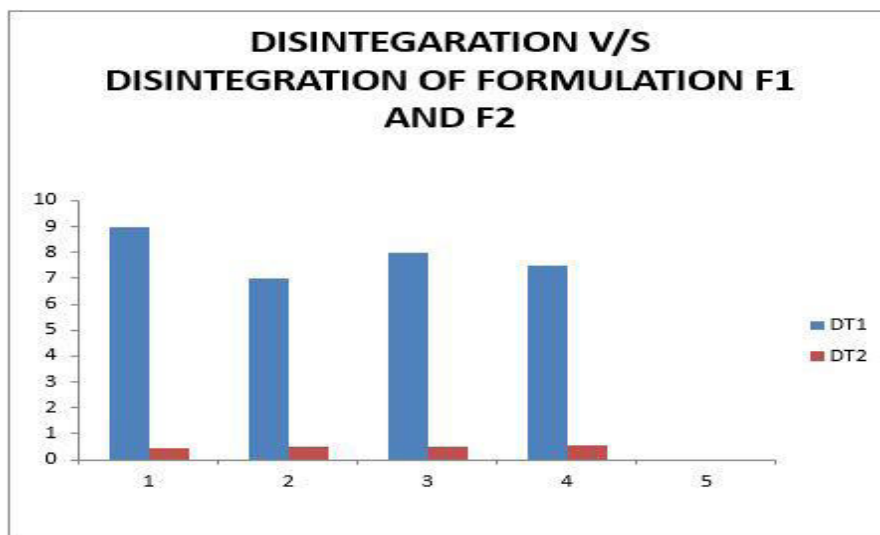


Figure 5: Graph Plot Of Disintegration 1 v/s Disintegration 2 of Formulation F1 And F2

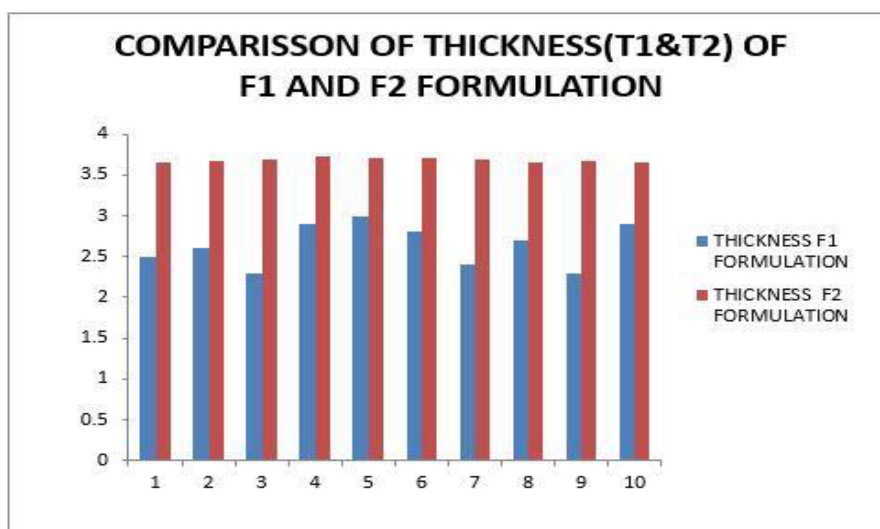


Figure 6: Effect of thickness on Formulation F1 and F2

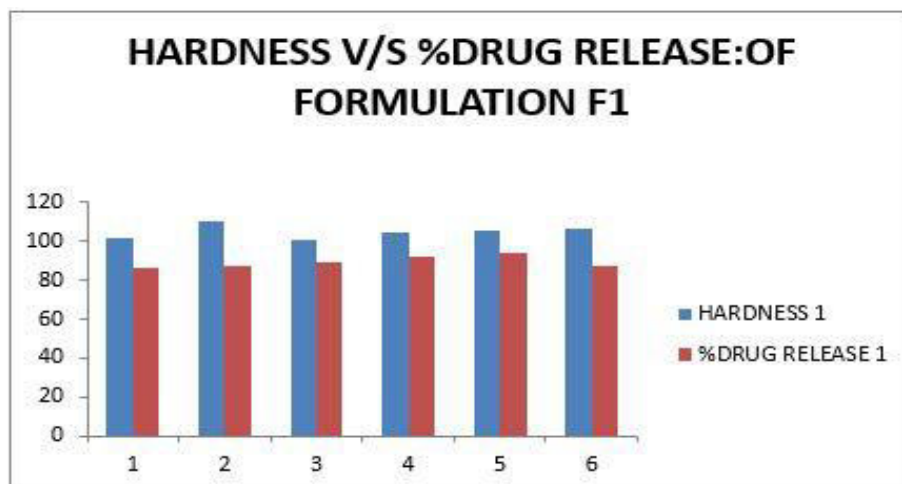


Figure 7: Formulation Graph Plot of hardness v/s drug release of F1 Formulation

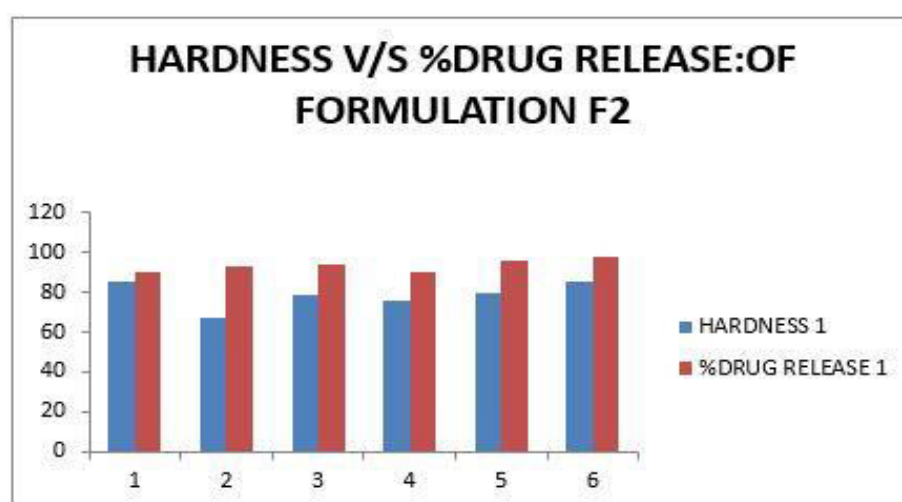


Figure 8: Formulation Graph Plot of hardness v/s drug release of F1 Formulation.

TABLES:

1	Pressure differential	(10-24 Pascal)
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Table 1: Environmental Conditions maintained for different formulations

Target	Range	Observed
Machine speed	Tablets/min	Tablets/min
Tablets/minutes	360-2880	1296

Table 2: Evaluation Parameters

Hardness tester	Parameter	Target	Range	Unit of Measurement	Valid sample
Dr. schleuniger pharmatron	Hardness	105N	50-100N	[N]	N=12

Table 3: Evaluation of Hardness with respect to reference range

Fluid Uptake	Hardness (H1)
F1 (15%)	102
	110
	105
	106
	104
	103
	101
	105
	102
	104
	Sum $(x_i)/n$ -104 X_{\max} 110 X_{\min} 101
Fluid Uptake	Hardness (H2)
F2 (13%)	67
	72
	79
	76
	72
	76
	85
	73
	72
	80
	Sum $(x_i)/n$ -75 X_{\max} -85 X_{\min} -67

Table 4: Fluid Uptake on Hardness of Formulation F1 And F2

S. No	F ₁	F ₂
1	W ₁ – 4.327g W ₂ – 4.326g = 0.02%	W ₁ - 4.411g W ₂ – 4.402g = 0.20%
2	W ₁ - 4.336 g W ₂ - 4.334g =0.05%	W ₁ -4.416 g W ₂ - 4.407g =0.20%
3	W ₁ - 4.394g W ₂ -4.493g =0.02%	W ₁ - 4.417 g W ₂ - 4.409g =0.18%
4	W ₁ - 4.388g W ₂ -4.385g =0.06%	W ₁ - 4.403 g W ₂ - 4.393g =0.22%
5	W ₁ – 4.387g W ₂ – 4.386g = 0.02%	W ₁ - 4.390g W ₂ – 4.387g = 0.20%
6	W ₁ - 4.336g W ₂ - 4.334g =0.05%	W ₁ - 4.398 g W ₂ - 4.380g =0.23%
7	W ₁ – 4.394g W ₂ -4.393g =0.02%	W ₁ - 4.391 g W ₂ - 4.381g =0.22%
8	W ₁ - 4.397g W ₂ -4.396g =0.02%	W ₁ - 4.403 g W ₂ -4.393g =0.22%
9	W ₁ - 4.390g W ₂ - 4.389g =0.02%	W ₁ - 4.405 g W ₂ - 4.394g =0.24%
10	W ₁ – 4.388g W ₂ -4.385g =0.06%	W ₁ - 4.396g W ₂ - 4.384g =0.27%

Table 5: Hardness on friability of f1 and f2 formulation

S.No	F1	F2	Hardness	
			H1	H2
1	Temp of water = 7.3 ⁰ C 9min	Temp of water =37.2 ⁰ C =00.45min	102	67
2	Temp of water = 37 ⁰ C 7 min	Temp of water =37.2 ⁰ C =00.48min	110	76
3	Temp of water = 36 ⁰ C 8 min	Temp of water =37.5 ⁰ C =00.51min	101	85

Table 6: Disintegration v/s Hardness of F1 Formulation

S.No	F1	F2
1	2.5mm	3.66mm
2	2.6mm	3.68mm
3	2.3mm	3.69mm
4	2.9mm	3.72mm
5	3.0mm	3.70mm
6	2.8mm	3.70mm
7	2.4mm	3.69mm
8	2.7mm	3.65mm
9	2.3mm	3.67mm
10	2.9mm	3.66mm

Figure 7: Effect of Hardness on Thickness of Formulation F1 &F2

Fluid Uptake	Hardness Range	% of Drug Release in 45 mint
	50-100	
F1(15%)	102	86
	110	87
	101	89
	104	92
	105	94
	106	87
	Sum (x_i)/n -104 X_{max} 110 X_{min} 101	Sum (x_i)/n X_{max} .94 X_{min} -86
Fluid Uptake	Hardness (H2) 50-100	% of Drug Release in 45 min 90-100
F2(13%)	85	90
	67	93
	79	94
	76	90
	80	96
	85	98
	Sum (x_i)/n -75 X_{max} -85 X_{min} -67	Sum (x_i)/n - X_{max} -98 X_{min} -90

Table: 8 Effect of Hardness v/s %Drug release

CONCLUSION:

From the experimental work we have observed that in the F1 formulation as the amount of water increases the hardness of the tablet increase and the corresponding friability decrease. Due to the increase in friability the disintegration time increases from the limit i.e. (NMT 7 min). As per the increase of hardness of the formulated tablet in F1 formulation, the corresponding %drug release decrease.

In case of F2 formulation, the amount of water decreases the hardness of the tablet and due that fact the friability as well as the disintegration of the F2 formulation also comes within the limits. Due to that

increase in the hardness the %drug release of the tablet in F2 formulation were also comes within the range.

So we are concluded that among the two formulated formulation, F2 formulation is fitted in all the criteria and is found the most suitable one.

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